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EFFECT OF DIFFERENT PLANT EXTRACTS AS ANTIFUNGAL AGENTS AGAINST ALTERNARIA ALTERNATA CAUSING LEAF BLIGHT DISEASE OF CABBAGE (BRASSICA OLERACEA)

ANSAR. A[1*] NARESH. L[1] AND SIMON. S.[2]

*[*1]Department of Molecular and Cellular Engineering, Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture, Technology & Sciences Deemed-to-be University.*
[2]Department of plant protection, Allahabad School of Agriculture, Sam Higginbottom Institute of Agriculture, Technology & Sciences Deemed-to-be University.

ABSTRACT

Plants historically have served as models in drug development. The production of medicines and the pharmacological treatment of diseases began with the use of herbs. The antifungal activity of five different plants namely, Aegle marmelos, Carica papaya, Mentha arvensis, Nicotiana plumbaginifolia and Tamarindus indica were tested against plant pathogenic fungi Alternaria alternata. The plant extracts were prepared in three solvents like acetone, methanol, and n-hexane. All the plants tested exhibited varying degrees of antifungal activity; however, the methanolic extract of Aegle marmelos showed maximum antifungal activity with the minimum growth zone of 7 mm at 24 hrs and 12 mm at 72 hrs as compared to control.

KEY WORDS: **Antifungal activity, Plant extract, Alternaria, acetone, methanol, n-hexane.**

ANSAR. A
Department of Molecular and Cellular Engineering, Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture, Technology & Sciences Deemed-to-be University.

***Corresponding author**

INTRODUCTION

Medicinal plants are gifts of nature to cure a limitless number of diseases among human beings[1]. The abundance of flora on the earth has led to an increasing interest in the investigation of traditional medicinal plants as potential sources of new antimicrobial agents[2]. Today, multiple drug resistance has become an alarming issue due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases[3]. Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages also post-harvest. There is a wide variety of fungal genera causing quality problems to plant produce that interfere aspect, nutritional value, organoleptic characteristics, and render limited shelf life[4]. Furthermore, residues of chemical pesticides in agricultural products cause damage to the health of animals, and humans. Considering the above facts there is an urgent need to search for alternative methods to manage the antibiotic resistance by developing antifungal and antibacterial compounds, which are eco-friendly and effective[5].Aegle marmelos L. commonly known as Bael belongs to the family Rutaceae. It is the only member of the monotypic genus Aegle. A. marmelos has been used from time immemorial in traditional systems of medicine for curing constipation, diarrhea, dysentery, peptic ulcer and respiratory infections. The leaves are useful in opthalamia, inflammations, diabetic and asthmatic complaints[6].Carica papaya a member from the family Caricaceae. C. papaya acts as a multi faceted plant with rich source of vitamins, antioxidants, flavanoids and polyphenols. Recently an antifungal chitinase gene has been cloned and characterized from papaya fruit[7].Mentha arvensis L. member of Lamiaceae, commonly known as cornmint, menthol mint or Japanese mint, was introduced into India in 1952 from Japan[8]. Plants from the genus Mentha are used for antimicrobial, antiviral and insecticidal activity. The plants are aromatic, stimulant and carminative. The infusion of leaves affords a remedy for rheumatism and indigestion. Mint is valued for its multipurpose uses in the field of pharmaceuticals, cosmetics as well as for flavoring foods, beverages and tobacco[9], anti-spasmodic, anti peptic ulcer agent, and has been given to treat indigestion, skin diseases, coughs and colds in folk medicine[10].Nicotiana plumbaginifolia Viv. (Solanaceae) commonly known a 'Wild Tobacco' is an annual or perennial weed herb with hairy stem, which was originated from Mexico and West Indies. In India, it is known to have medicinal properties evidenced by antispasmodic, diuretic, expectorant and is widely used in the treatment of several human ailments like rheumatic, swelling in order to relieve the pain, dried leaves are used in the treatment of nausea and travel sickness[11].Tamarind (Tamarindus indica) "Indian date" is a leguminous tree in the family Fabaceae indigenous to tropical Africa. The genus Tamarindus is a monotypic taxon, having only a single species. The pharmacological investigations revealed that the plant pulp is antibacterial, antifungal, hypoglycemic, cholesterolemic, cytotoxic antioxidative, laxative, antiinflammatory, gastrointestinal, and hypolipomic in its activities[12]. Alternaria fungus has about one hundred species, found in various places all over the world. Many of them are plant pathogens and cause disease in a wide range of hosts. Alternaria alternata has an important place among species of this genus, as it depends on range of hosts including garden plants, field crops, vegetables, and ornamentals. The taxon is the principal causative agent of black mould of ripe tomatoes[13], brown spot on citrus[14], brown necrotic lesions on foliage, black pit disease of potatoes[15] and black rot of harvested fruits. Considering the antimicrobial potential of plants with reference to antifungal and antibacterial agents, a systematic investigation was undertaken to screen the local flora for antifungal activity of Aegle marmelos, Carica papaya, Mentha arvensis, Nicotiana plumbaginifolia and Tamarindus indica against the fungus Alternaria alternata.

MATERIALS AND METHODS

Sample collection

The fresh, mature healthy, leaves of M. arvensis and N. plumbaginifolia were collected from Forestry department, Sam Higginbottom Institute of Agriculture Technology & Sciences, Allahabad and A. marmelos, C. papaya, and T. indica were collected from Khushru Bagh Garden, near Railway junction, Allahabad.

Sterilization of plant material

The disease free and fresh plants were selected. Leaves were surface sterilized by washing under tap water and distilled water and shade dried at room temperature. About 500g of fresh leaves were taken for solvent extraction.

Preparation of plant extraction

Dried leaves were powdered by using blender, and the powder was used for preparation of extracts in three organic solvents, viz. acetone, methanol and n-hexane. The sample (powder) 100 g each was dissolved in 400 ml respective solvent using soaking method and allowed to stand at room temperature for 7 days. The extracts obtained with different solvents were filtered through Whatman filter paper No. 1. To obtain crude extract samples were kept on water bath (at 40ºC) until complete solvent was evaporated. The final yield of crude extract (1 g) was re-suspended in 20% tween-20 (4 ml); this was used as stock solution and kept at 4 °C for subsequent use.

Collection and isolation of the pathogen

The leaves of the infected cabbage plants bearing characteristic symptoms (concentric rings) of leaf blight disease were collected from Forestry department, Sam Higginbottom Institute of Agriculture Technology & Sciences, Allahabad. The infected leaf bits were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 30 seconds, washed repeatedly with sterile distilled water to remove the traces of mercury if any and transferred in sterilized petri plates (1-2 leaf bits each) containing potato dextrose agar (PDA) media. The petri plates were incubated at room temperature for the growth of Alternaria. After 3 days, blackish colony growth was observed in incubated petri dishes.

Identification of Isolated Fungi[16]

The fungal isolate from growing culture was identified by lactophenol cotton blue staining. Microscopic examination was carried out after examining the colony characteristics, while the morphological and cultural characteristics were observed. The test fungus were grown and maintained on potato dextrose agar slants, following incubation for 5 days, the cultures were either utilized or stored until required. The organisms were sub cultured to obtain pure colonies and it was done once in every 15 days.

Antifungal assay of plant extracts[17]

The test was carried out on PDA agar plates using the pour plate technique. A volume of 1 ml of each extract (acetone, methanol and n-hexane) was aseptically poured in respective petri-plates followed by the addition of 9 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents. A petri-plate with PDA having no plant extract was used as control. After the solidification of the media, pure culture of the isolated fungi was then transferred aseptically onto the petriplates with plant extract using a sterile cork borer of 5.0 mm diameter upside down right at the centre. The petri plates were incubated at 27°C and growth (diameter) of the tested fungi was measured after 24 hrs and 72 hrs respectively.

RESULTS AND DISCUSSION

Antifungal activity of plant extract

Antifungal activity of selected plants was evaluated by pour plate technique. The results obtained after conducting the experiment were as follows (Table 1). Among all the five plants, the methanol extract of A. marmelos had a growth zone of 7 mm (24 hrs) and 12 mm (72 hrs), and the n-hexane extract of N. plumbaginifolia had a growth zone of 8mm (24 hrs) and 11 mm (72 hrs) exhibited maximum antifungal activity against Alternaria alternata when compared with other plant extracts. The

methanol extract of M. arvensis showed prominent antifungal activity with growth zone of 7mm (24 hrs) and 15mm (72 hrs). In C. papaya, the acetone extract inhibits the growth of A. alternata with growth zone of only 10 mm (24 hrs) and 19 mm (72 hrs). Acetone extract of Tamarindus indica was effective to show antifungal activity with the growth zone, minimum of 11 mm (24 hrs) and a maximum of 23 mm after 72 hrs.

Table1
Antifungal activity of the plant(s) extracts against Alternaria alternate

Plant	Extract	Control growth of fungi (mm.)	
		24hrs	72hrs
Aegle marmelos	Acetone	08	16
	Methanol	07	12
	n-Hexane	10	20
	Control	14	33
Carica papaya	Acetone	10	19
	Methanol	09	22
	n-Hexane	11	23
	Control	14	33
Mentha arvensis	Acetone	08	15
	Methanol	07	15
	n-Hexane	07	16
	Control	11	27
Nicotiana plumbaginifolia	Acetone	10	19
	Methanol	07	18
	n-Hexane	08	11
	Control	11	27
Tamarindus indica	Acetone	11	23
	Methanol	12	26
	n-Hexane	12	27
	Control	14	33

Evidently Maity reported that crude extract of Aegle marmelos has therapeutic potential in the treatment of many microbial diseases, diabetes and gastric ulcers[18]. Methanol extracts of A. marmelos has shown strong activity against multi-drug resistance Salmonella typhi. Similarly the methanol leaf extracts of various medicinal plants showed significant antibacterial and antifungal activity against Aspergillus flavus, Dreschlera turcica and Fusarium verticillioides[19].Kumar investigated that C. papaya has antifungal medicinal properties[20]. The effects of different concentrations of alcoholic extract of C. papaya (root, shoot and seed) on the radial growth of plant against the pathogenic fungi viz. Aspergillus niger, Aspergillus flavus, Candida albicans and Microsporum fulvum revealed that with the increase in concentrations the rate of growth inhibition also increases. This suggests the notion that C. papaya acts as antifungal agent against different fungi. This study is in accordance with the present work where different solvents were used to assess the antifungal potential of C. papaya. Recently an antifungal chitinase gene has been cloned and characterized from papaya fruit[7]. The aqueous and methanol extract of N. plumbaginifolia showed strongest anti-microbial activity against Bacillus fusiformis[10]. In addition to this, similar results were observed in the present study, which showed that N. plumbaginifolia has antifungal properties also. The ethanolic extract of T. indica leaves and pulp had antifungal activity against Aspergillus niger, Aspergillus flavus along with Fusarium oxysporium[21]. T. indica could be a promising antifungal agent and can be used as traditional medicine for the treatment of fungal infections. In another study the three solvents (aqueous, ethanol and acetone) used for T. indica extraction, the acetone extracts showed highest antimicrobial activity against some common gram negative and gram positive bacteria and fungi, followed by the ethanol extracts and water extracts[22]. Acetone extracts might have

had higher solubility for more phytoconstituents. These facts were in accordance with the results of the present work, which illustrated the significant anti-mycolytic effect of T. indica acetonic extract against A. alternata.

SUMMARY AND CONCLUSION

Medicinal plants have attracted the attention of scientists all over the world. Bioactive compounds derived from natural sources could be a potential candidate as an antifungal agent, especially in the present scenario where human and plant fungal pathogens have adopted resistance against antifungal antibiotics. The results obtained from the study clearly suggest that selected plant species have antifungal properties against Alternaria alternata. The present investigation is an important step in developing plant based pesticides and drugs, which are eco-friendly for the management of the pathogenic fungi and bacteria.

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